CHALMERS
UNIVERSITY OF TECHNOLOGY

Viral interference cannot be concluded from datasets containing only symptomatic patients

Viral interference cannot be concluded from datasets containing only symptomatic patients

In the study by Anchi Wu and colleagues,[1] the authors claim that rhinovirus reduces susceptibility to influenza A virus through a mechanism known as viral interference, basing their conclusions on viral detection rates in clinical samples and in-vitro experiments. We agree that viral interference might contribute to a reduction in the number of co-infections. However, this conclusion cannot be drawn from their data, as calculating the expected number of co-infections in a sample of non-randomly selected patients is not statistically accurate.

To exemplify this point, assume that two viruses, X and Y, both with a prevalence of infection of 2%, were independently present in a population of 10 000 individuals, and that all individuals had samples taken on the same day. We then expect to find $10\,000 \times 0{\cdot}02 \times 0{\cdot}02=4$ individuals infected with both viruses, and 196 individuals each infected with either X or Y viruses. Further assume that, of 800 individuals with symptoms, 404 carry pathogens other than X or Y. Supplementary figure 1A (appendix) shows contingency tables with the expected number of individuals in the whole population and in the symptomatic population infected with X, Y, X and Y, or neither. Calculation of the expected number of individuals co-infected with X and Y from the contingency table of symptomatic individuals gives an expected number of $200 \times 200/800=50$. Such a calculation leads to the conclusion that the observed number (n=4) is lower than the expected number (n=50); the odds ratio would be 0·042.

This approach was applied in the study by Wu and colleagues,[1] in which they collected data only from patients with symptoms, and calculated the expected number of individuals co-infected with rhinovirus and influenza A virus as $989 \times 922/13\,707=67$. Based on this calculation, they concluded that the observed number of co-infections (n=12) was significantly lower than the expected number (n=67). As shown in the above example, this conclusion is not justified, and the mistake illustrates that calculating the expected number of co-infections from observations in datasets including only individuals with symptoms is misleading, as pathogens that are truly independent in the population by such a calculation will appear to be negatively associated with each other (ie, occur as a co-infection less often than expected).

To provide another example, we compared data from the study by Wu and colleagues[1] with that of an ongoing study of 8178 samples referred for testing of respiratory pathogens by a multiplex PCR panel at our centre, over the course of 1 year. As shown in supplementary figure 1B and C (appendix), negative associations were observed when the co-infection frequency was compared with the frequency of infection with each virus alone, not only for rhinovirus and influenza A, but also for several virus pairs. Although Wu and colleagues[1] concluded that viral interference was responsible for these negative associations (at least for rhinovirus and influenza A), we consider that these results reflect what is statistically anticipated. Importantly, we notice that these considerations were also missing from another study on the same topic published in 2019.[2]

Our comment on the interpretation of the clinical data in the study by Wu and colleagues[1] does not argue against the existence of viral interference. However, a study published in 2019 showed that influenza A blocked rhinovirus replication, but that rhinovirus did not interfere with influenza A virus replication in vitro.[3] These results contradict the in-vitro data presented by Wu and colleagues,[1] and these opposing conclusions highlight the difficulties in translating experimental observations to a clinical setting.

We declare no competing interests.

*Rickard Nordén, *Magnus Lindh, Staffan Nilsson, Johan Westin*
magnus.lindh@microbio.gu.se

Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg 41346, Sweden (RN, ML, JW); Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden (SN)

1 Wu A, Mihaylova VT, Landry ML, Foxman EF. Interference between rhinovirus and influenza A virus: a clinical data analysis and experimental infection study. *Lancet Microbe* 2020; **1:** e254–62.
2 Nickbakhsh S, Mair C, Matthews L, et al. Virus–virus interactions impact the population dynamics of influenza and the common cold. *Proc Natl Acad Sci U S A* 2019; **116:** 27142–50.
3 Essaidi-Laziosi M, Geiser J, Huang S, Constant S, Kaiser L, Tapparel C. Interferon-dependent and respiratory virus-specific interference in dual infections of airway epithelia. *Sci Rep* 2020; **10:** 10246.

See **Online** for appendix